

PTEN Methylation Dependent Sinonasal Mucosal Melanoma

Sang Hee Lee, MD¹
Mi Ryung Roh, MD, PhD¹
Beodeul Kang, MD^{2,3}
Kyu Hyun Park, PhD^{2,3}
Soo Hee Kim, MD⁴
Sang Eun Lee, MD, PhD¹
Sun Young Rha, MD, PhD^{2,3,5}

¹Department of Dermatology,
Severance Hospital, Cutaneous Biology
Research Institute, ²Yonsei Song-Dang
Institute for Cancer Research,
³Division of Medical Oncology,
Department of Internal Medicine,
⁴Department of Pathology,
⁵Brain Korea 21 Plus Project for
Medical Science, Yonsei University
College of Medicine, Seoul, Korea

Correspondence: Sun Young Rha, MD, PhD
Division of Medical Oncology,
Department of Internal Medicine,
Yonsei Song-Dang Institute for Cancer Research,
Yonsei Cancer Center, Yonsei University
College of Medicine, 50-1 Yonsei-ro,
Seodaemun-gu, Seoul 03722, Korea
Tel: 82-2-2228-8130
Fax: 82-2-393-3652
E-mail: rha7655@yuhs.ac

Received December 15, 2014
Accepted January 24, 2015
Published Online March 18, 2015

Sinonasal mucosal melanoma (SMM) is an aggressive and rare type of melanoma. Although the classic RAS-RAF-MEK pathway is thought to be the main pathway involved in melanoma pathogenesis, genetic alterations in the phosphatidylinositol 3-kinase-AKT pathway, including *PTEN*-regulated signaling, are also thought to contribute. So far, data regarding altered *PTEN* expression and epigenetic mechanism of *PTEN* silencing in development of SMM is extremely limited. Herein we report on a case of SMM with liver and bone metastases with an epigenetic alteration of *PTEN*. Results of mutation analysis for *BRAF*, *NRAS*, *HRAS*, *KRAS*, *PIK3CA*, *c-Kit*, and *PTEN* were negative; however, methylation of *PTEN* CpG islands was observed. Our case not only supports *PTEN* as a major tumor suppressor involved in melanoma tumorigenesis, but also a potential epigenetic mechanism of *PTEN* silencing in development of SMM.

Key words

Methylation, Phosphatase and tensin homolog,
Sinonasal melanoma

Introduction

Sinonasal mucosal melanoma (SMM) is an extremely rare tumor, accounting for less than 1% of all western melanomas [1]. The general incidence of SMM in Asia is poorly understood and under-represented in the English literature. It is a mucosal melanocytic neoplasm characterized by a high tendency to recur and metastasize to various distant sites. The

molecular-driven stepwise transformation of melanocytes has been extensively documented in melanoma, however, much less is known about the rarer and more aggressive forms of primary mucosal melanoma, including SMM.

Aberrant activation of the mitogen-activated protein kinase (MAPK) pathway has been reported in 70% of melanoma, and it has been considered that the MAPK pathway is the major signaling pathway involved in melanoma pathogenesis. Mutations in proteins along the RAS-RAF-MEK

pathway have been well documented in melanoma, with diverse variations according to its subtypes (63% BRAF, 26% NRAS, and 3% KIT in overall) [2]. Recently, therapies targeting mutations of the MAPK pathway have brought significant survival improvement, particularly with the BRAF inhibitor targeting V600E BRAF mutation [3]. However, there are still a significant number of patients without mutations of the MAPK pathway, in whom treatment can be extremely challenging. Greater clarification of additional or unknown genetic alterations in other signaling pathways is needed, as they may be exploited as new therapeutic targets.

In addition to the MAPK pathway, the phosphatidylinositol 3-kinase (PI3K)–AKT pathway is also considered to have a critical role in the pathogenesis of melanoma. Deletion or mutation of phosphatase and tensin homologue (*PTEN*), a tumor suppressor gene at 10q23.3, has been reported in a variety of advanced tumors including malignant melanoma. The *PTEN* gene encodes a phosphatase that degrades the product of PI3K [4]. Hence the loss of function of *PTEN* from tumor cells causes accumulation of critical messenger lipids, which in turn increase AKT phosphorylation and activity, leading to decreased apoptosis and increased mitogen sig-

naling. Because *PTEN* protein is low to absent in a significant number of melanomas whereas mutations and deletions of *PTEN* gene vary widely from 10% to 60% [4], speculation that *PTEN* inactivation might occur through a mechanism other than mutation, such as epigenetic silencing, has emerged. Aberrant CpG island methylation of *PTEN* has been identified as an alternative mechanism of *PTEN* inactivation in various type of cancers including lung cancer, endometrial carcinoma, prostate cancer, brain tumors, cervical neoplasm, malignant melanoma, hematologic malignancies and nasopharyngeal carcinoma [5]. Due to the low incidence of SMM, study of the molecular abnormalities relevant to the biology of this melanoma subtype has been limited [6,7]. In particular the mechanisms of *PTEN* silencing are little understood. In fact, epigenetic silencing of *PTEN* in sinonasal type of melanoma has not been reported previously. Herein we report on a case of SMM in a 53-year-old woman which showed reduced *PTEN* expression with methylation of the *PTEN* promoter, suggesting that epigenetic *PTEN* silencing is one of the possible mechanisms involved in SMM development.

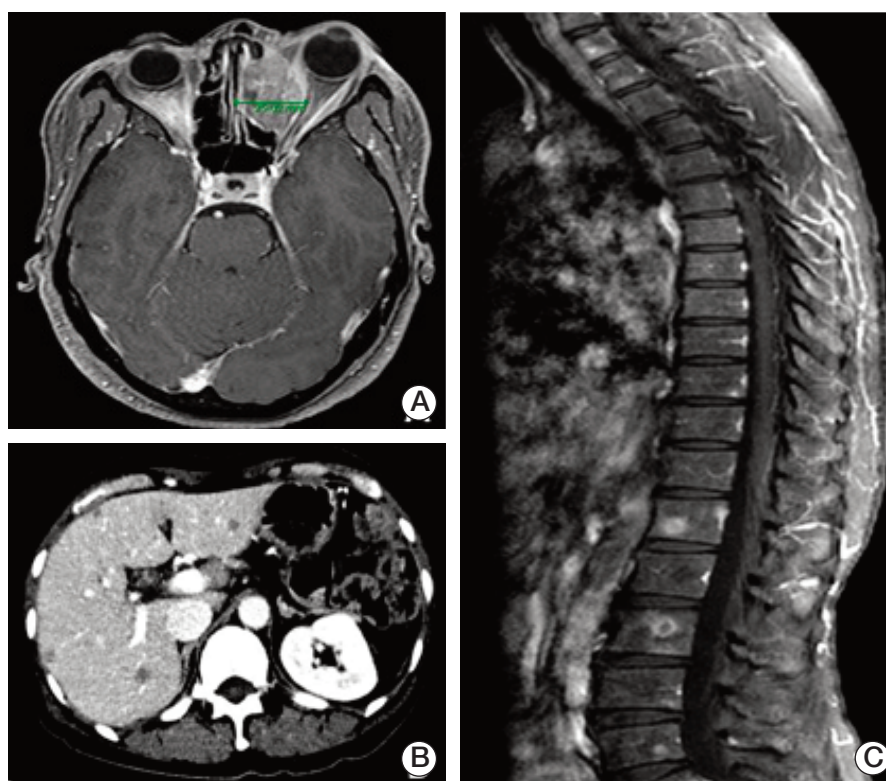


Fig. 1. Imaging analysis of the patient. (A) Magnetic resonance imaging (MRI) of the paranasal sinuses shows an enhancing soft tissue lesion in the left frontoethmoid sinus extending to the left orbit. (B, C) Multiple metastases are seen in liver and vertebrae in the abdominopelvic computed tomography and spine MRI scan.

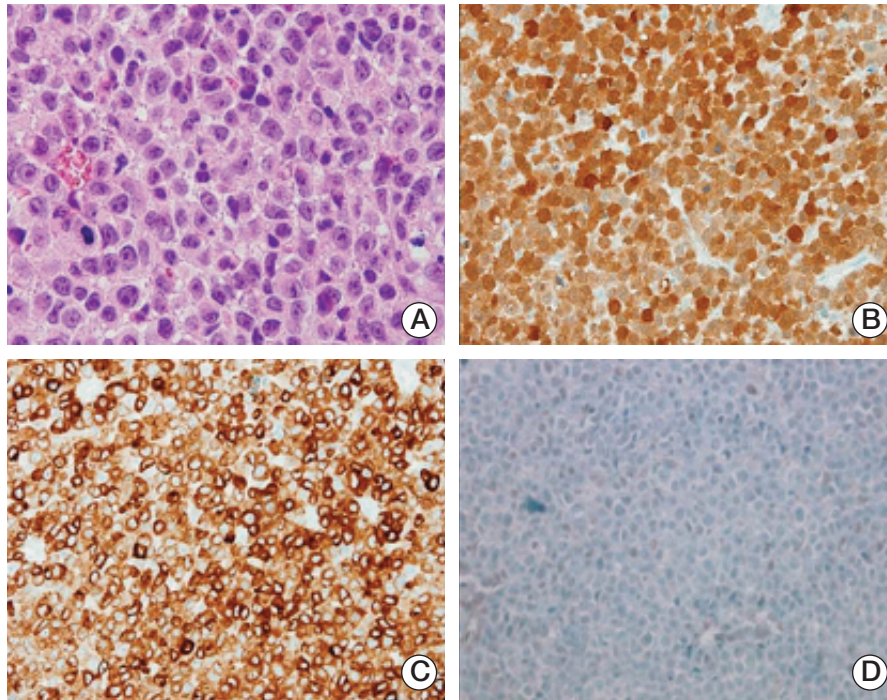


Fig. 2. Pathologic findings of the biopsy specimen obtained from the nasal cavity mass. (A) Round tumor cells with atypia, showing pleomorphic and hyperchromatic nuclei with prominent nucleoli are seen (H&E staining, $\times 400$). (B-D) Immunohistochemistry staining shows positive staining in the tumor cells for S-100 (B) and Melan A (C) (B and C, $\times 200$), and decreased level of PTEN protein expression (D, $\times 200$).

Case Report

A 53-year-old woman presented with complaints of a left orbit mass with orbital swelling. The patient had symptoms of diplopia and intermittent headache for 2 months. There was no relevant past medical or family history. Magnetic resonance imaging (MRI) scan of the sinuses showed an enhancing soft tissue lesion in the left frontoethmoid sinus extending to the left orbit and anterior cranial fossa (Fig. 1A) with another enhancing lesion observed at the left sphenoid ridge. Nasopharyngeal biopsy of the mass showed round tumor cells with atypia, showing pleomorphic and hyperchromatic nuclei with prominent nucleoli (Fig. 2A). In immunohistochemical staining, tumor cells were positive for S-100, Melan A, human melanoma black 45, Fli-1, and CD99 resulting in the diagnosis of malignant mucosal melanoma (Fig. 2B and C).

Further imaging evaluation with abdominopelvic computed tomography (CT) and spine MRI revealed multiple hepatic and bone metastases (Fig. 1B and C). MRI of the spine showed bony metastases in the C2, C7, and T10 vertebrae, and abdominopelvic CT showed innumerable metastatic

nodules in the both lobes of the liver.

For genetic analysis, genomic DNA was extracted from a formalin-fixed, paraffin-embedded tissue block using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). Pyrosequencing was performed using a PyroMark Q24 (Qiagen, Germantown, MD); mutations for *BRAF*, *NRAS*, *HRAS*, *KRAS*, *PIK3CA*, *GNAQ*, *GNA11*, and *EGFR* were not found [8]. *KIT* copy number was also assessed using a QuantiTect SYBR Green PCR Kit (Qiagen) and calculated according to a previously described method [9], which revealed lack of *KIT* amplification. Agarose gel electrophoresis of *PTEN* PCR products following the previously described method [10] showed a band of *PTEN* product in the patient sample compared to control cell line SK-MEL-24 with *PTEN* deletion, demonstrating the absence of *PTEN* deletion (Fig. 3A). The methylation status of the *PTEN* promoter region was additionally examined by pyrosequencing using bisulfite-modified DNA, which revealed methylations of five *PTEN* CpG island sites (Fig. 3B). Subsequent immunohistochemical staining of PTEN protein (rabbit polyclonal antibody, Invitrogen, Carlsbad, CA) in the biopsy specimen showed decreased expression of *PTEN* in the tumor cells (Fig. 2D). Finally, the patient was diagnosed as SMM with

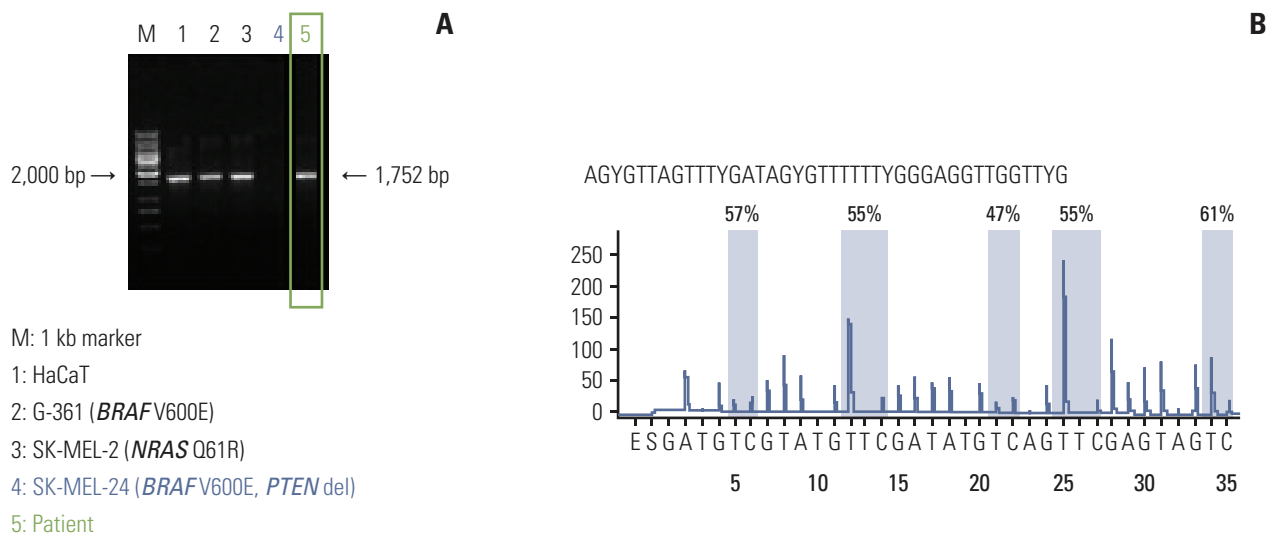


Fig. 3. Examination of *PTEN* polymerase chain reaction (PCR) product and methylation status of *PTEN* promoter gene. (A) Agarose gel electrophoresis shows loss of *PTEN* PCR product in the SK-MEL-24 cell line, which is known to have *PTEN* deletion, while the patient sample shows a band of *PTEN* product. (B) Methylation status of the *PTEN* promoter examined by pyrosequencing using bisulfite-modified DNA shows methylation of five different CpG island sites in the patient's genomic DNA.

multiple liver and bone metastases, with an epigenetic alteration of *PTEN* promoter.

As the patient was not considered amenable for surgical excision due to the presence of distant metastases, she was treated with palliative chemotherapy with dacarbazine. MRI imaging after the fourth cycle showed partial response to systemic treatment, showing decrease in the size of the primary orbital lesion and multiple metastatic sites. However after the sixth cycle, the patient showed rapid progression of the disease with leptomeningeal seeding and increased size and number of liver and bone metastases. The patient eventually expired of progressive disease 6 months after the initial diagnosis.

Discussion

SMM is a rare type of malignant melanoma, accounting for less than 1% of all western melanomas and less than 5% of all sinonasal tract neoplasms [1]. Although SMM accounts for only a small fraction of melanoma, the incidence continues to increase by 6% to 7% every year [11]. Symptoms at presentation are not specific and the diagnostic latency can be long [12]. Although only a few data are available regarding the genetic alterations in SMM, molecular studies reported so far have demonstrated that these tumors only

rarely harbor *BRAF* mutation [6,7]. Prognosis remains poor, unlike other subtypes of melanoma which now have better survival rates due to the development of newer immunologic and molecularly targeted agents (anti-CTLA-4, anti-PD-1 antibodies, and *BRAF* inhibitors) [3]. In light of these, the molecular targets in the pathogenesis of SMM need to be clarified as it could ultimately lead to better treatment outcome.

Although the precise pathogenetic mechanism in the development of SMM is unclear, the malignant transformation of melanocytes is thought to occur through sequential accumulation of genetic and molecular alterations. Given that the alterations in intracellular signaling cascades of MAPK and PI3K-AKT pathways contribute to the pathogenesis of melanoma, genetic aberrations of *BRAF*/*NRAS*/*HRAS*/*KRAS*, *PIK3CA*, *c-Kit*, and *PTEN* were examined in our patient. No mutation, amplification, or deletion of the candidate genes was found in the patient sample, whereas hypermethylation of the *PTEN* promoter was found.

Sporadic melanomas may have loss of *PTEN* through loss of heterozygosity, deletion, or mutation [1]. On the other hand, epigenetic *PTEN* silencing without *PTEN* mutation has also been studied. In one study with 34 melanomas, 61% of the specimens showed reduced or absent *PTEN* expression but neither *PTEN* mutation nor deletion was observed (11 of 18, 61%), suggesting an epigenetic mechanism of biallelic functional inactivation of *PTEN* [13]. Another study including 37 sera from melanoma patients showed positional

methylation of CpG islands in the *PTEN* gene, which leads to reduced *PTEN* expression [4]. In cutaneous melanomas, a direct relationship between *PTEN* hypermethylation and its decreased expression has been demonstrated [14]. Collectively, epigenetic *PTEN* silencing appears to be a relevant mechanism that may contribute to the constitutive activation of the AKT pathway in melanoma tumorigenesis.

In SMM, a significant portion of specimens (13 of 27, 48.1%) has shown loss of *PTEN* by immunohistochemistry [6]. However, compared to other melanomas, epigenetic *PTEN* silencing for this particular melanoma subtype has not been studied before, possibly due to its low incidence. Our data suggest that the reduced *PTEN* expression in our SMM patient was due to the aberrant methylation of *PTEN* promoter gene. As loss of function mutations in *PTEN* occur in only a fraction of *PTEN*-deficient tumors, *PTEN* expression may be lost by various other mechanisms including genomic deletion, epigenetic and transcriptional silencing. Therefore, it is necessary to determine *PTEN* status in tumors by protein quantification, DNA sequencing and the methylation status of the CpG islands of *PTEN*, considering that the mechanisms of epigenetic silencing are increasingly being recognized in melanoma.

Data on the role of *PTEN* genes in the process of melanoma cell transformation and tumor progression are still inconclusive. In cutaneous melanoma, *PTEN* methylation has been related to increased mortality [14]. However, in another study, decreased *PTEN* expression showed correlation with ulceration, but not with survival in melanoma patients [15]. Although there are contrasting results, the need to examine alterations of *PTEN* and their clinical relevance seems increasingly important.

The ideal treatment of SMM remains obscure and no definitive conclusions have been reached. Surgical excision continues to be the mainstay for treatment of primary SMM. For poor surgical candidates, targeted therapy, radiotherapy and chemotherapy can be considered. So far, treatment options are limited for patients without *BRAF* mutations. Response rate of chemotherapy including dacarbazine and other cytotoxic agents is approximately 10% to 20%, irrespec-

tive of the different regimens used [1]. Chemotherapy fails to prolong survival and is often used as palliative treatment for those with advanced disease [1]. In our patient, palliative dacarbazine alone was administered, and partial response was noted with improved patient's quality of life. However, progression of the disease eventually occurred after the sixth cycle. The 5-year overall survival rate for SMM ranges from 17.1% to 35.1% [1], and is reported to be even lower in patients with metastasis [12].

To the best of our knowledge this is the first case of SMM to show decreased *PTEN* expression due to methylation of *PTEN* promoter gene. Our case additionally supports *PTEN* as one of the major tumor suppressor involved in melanoma tumorigenesis and also the possibility of the epigenetic mechanism of *PTEN* silencing in development of SMM. However, it should be emphasized that more case studies examining the methylation of *PTEN* promoter status in SMM will be needed to support the presumption. Further comparative studies of *PTEN* methylation status in normal mucosal tissue, benign sinonasal tumors and SMM should be conducted to determine the precise role of epigenetic *PTEN* silencing in SMM.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea, which is funded by the Ministry of Education, Science, and Technology (2011-0022376).

References

- Gavriel H, McArthur G, Sizeland A, Henderson M. Review: mucosal melanoma of the head and neck. *Melanoma Res.* 2011;21:257-66.
- Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, et al. A landscape of driver mutations in melanoma. *Cell.* 2012;150:251-63.
- Sondak VK, Gibney GT. Indications and options for systemic therapy in melanoma. *Surg Clin North Am.* 2014;94:1049-58.
- Mirmohammadsadegh A, Marini A, Nambiar S, Hassan M, Tannapfel A, Ruzicka T, et al. Epigenetic silencing of the *PTEN* gene in melanoma. *Cancer Res.* 2006;66:6546-52.
- Li J, Gong P, Lyu X, Yao K, Li X, Peng H. Aberrant CpG island methylation of *PTEN* is an early event in nasopharyngeal carcinoma and a potential diagnostic biomarker. *Oncol Rep.*

- 2014;31:2206-12.
6. Turri-Zanoni M, Medicina D, Lombardi D, Ungari M, Balzarini P, Rossini C, et al. Sinonasal mucosal melanoma: molecular profile and therapeutic implications from a series of 32 cases. *Head Neck*. 2013;35:1066-77.
 7. Zebary A, Jangard M, Omholt K, Ragnarsson-Olding B, Hansson J. KIT, NRAS and BRAF mutations in sinonasal mucosal melanoma: a study of 56 cases. *Br J Cancer*. 2013;109:559-64.
 8. Si L, Kong Y, Xu X, Flaherty KT, Sheng X, Cui C, et al. Prevalence of BRAF V600E mutation in Chinese melanoma patients: large scale analysis of BRAF and NRAS mutations in a 432-case cohort. *Eur J Cancer*. 2012;48:94-100.
 9. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) method. *Methods*. 2001;25:402-8.
 10. Sandell S, Schuit RJ, Bunyan DJ. An intronic polymorphic deletion in the PTEN gene: implications for molecular diagnostic testing. *Br J Cancer*. 2013;108:438-41.
 11. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005;55:74-108.
 12. Sun CZ, Li QL, Hu ZD, Jiang YE, Song M, Yang AK. Treatment and prognosis in sinonasal mucosal melanoma: a retrospective analysis of 65 patients from a single cancer center. *Head Neck*. 2014;36:675-81.
 13. Zhou XP, Gimm O, Hampel H, Niemann T, Walker MJ, Eng C. Epigenetic PTEN silencing in malignant melanomas without PTEN mutation. *Am J Pathol*. 2000;157:1123-8.
 14. Lahtz C, Stranzenbach R, Fiedler E, Helmbold P, Dammann RH. Methylation of PTEN as a prognostic factor in malignant melanoma of the skin. *J Invest Dermatol*. 2010;130:620-2.
 15. Mikhail M, Velazquez E, Shapiro R, Berman R, Pavlick A, Sorhaindo L, et al. PTEN expression in melanoma: relationship with patient survival, Bcl-2 expression, and proliferation. *Clin Cancer Res*. 2005;11:5153-7.